Mechanism of palindrome-induced DNA double stranded breakage (DSBs) in yeast

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Newsha Tavakoli

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Mechanism of palindrome-induced DNA double stranded breakage (DSBs) in yeast

Approved by:

Dr. Kirill Lobachev, Advisor
School of Biology
Georgia Institute of Technology

Dr. Francesca Storici
School of Biology
Georgia Institute of Technology

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
GCR	Gross chromosomal rearrangement
DSB	Double strand breakage
YPD	Yeast extract peptone dextrose
UV	Ultra violate

SUMMARY

Chromosomes are dynamic cellular structures that carry genetic information; they combine the stability required for inheritance and the flexibility required for change. The departure from normal chromosome number and arrangement is underlying molecular feature of many cancers and hereditary diseases in humans. Gross chromosomal rearrangements (GCR), which can be caused by DNA double strand breakage (DSB), is a common type of chromosomal mutations [Inagaki et al., 2013]. Long palindromic sequences that are self-complementary DNA sequences and capable of forming non-B cruciform structures are one of the recognized breakpoint hotspots [Inagaki et al., 2013]. Genomic instability of palindromic DNA can be induced by some reactions that cleave the cruciform structure diagonally at the four-way junction, leading to frequent DNA breakage [Inagaki et al., 2013]. Currently, the enzymes that cause these DSBs in inverted repeats is still under questions even though there are studies showed multiple structure-specific nucleases can potentially target hairpin or cruciform structure [Schwartz, 2017], and this research aims to identify the enzyme by screening for mutants with lower frequency of DSB in yeast population that has been treated with mutagenic agent ethyl methanesulfonate (EMS). First, mutants exhibiting decreased levels of GCRs were identified. Then, hypo-GCR isolates were tested with Southern blotting to reveal possible low DSB mutants. These mutants will further be tested in comparison to the wild-type to identify the nuclease that causes DSB in palindromic DNA of yeast cells.

CHAPTER 1

INTRODUCTION

Gross chromosomal rearrangemenst (GCR), which can be caused by DNA double strand breakage (DSB), is a common type of chromosomal mutations [Inagaki et al., 2013]. Breakpoint hotspots are regions in chromosome more susceptible to DNA breakage [Inagaki et al., 2013]. Long Inverted repeats or palindromic sequences are one of the recognized GCR breakpoint hotspots [Inagaki et al., 2013]. An inverted repeat is a sequence of DNA that are identically repeated in the opposite strand, but in the opposite direction (figure 1, A) [Smith, 2008]. A strand of DNA in an inverted repeat is able to pair with itself, and form secondary structures such as hairpins and cruciform structures (figure 1, B) [Smith, 2008]. A hairpin is a structure that forms when only one strand of DNA is involved and a cruciform structure forms when both strands of palindromic DNA are involved in intrastrand binding [Smith, 2008].

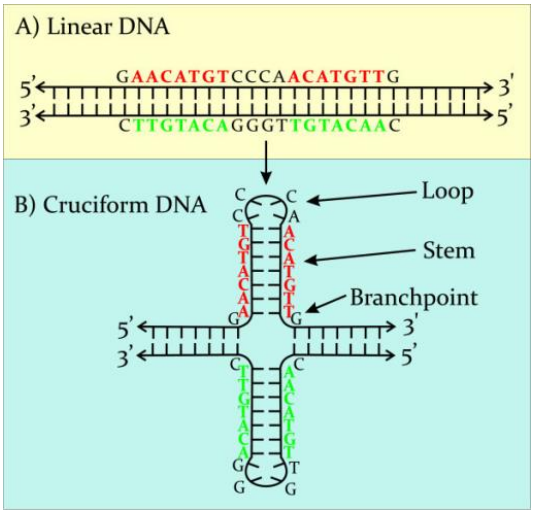

Figure 1, Inverted repeat DNA [Brázda, 2011]

Leading cause of DSB in palindromic DNA

Previous research shows that genomic instability of palindromic DNA is a result of certain endonucleases cleaving the cruciform structure diagonally at the four-way junction, leading to frequent DNA breakage [Inagaki et al., 2013].

Inverted repeats have different levels of instability. The breakage rate is not only determined by the nature of inverted repeats, such as symmetry, length, etc, [Lobachev, 1998] but also controlled by an external gene that causes instability of inverted repeats,

and therefore, it can be concluded that the frequent breakage of palindromic DNA is facilitated/caused by an enzyme [Lu, 2015]. However the list of enzymes that cause these DSBs at inverted repeats is still undetermined.

Research Goals

This research inspects the enzyme that is responsible for inverted repeats DSB by investigating the frequency of DSB in yeast wild-type cell and mutants. The wild-type yeast cell used in this research has a high frequency of DSB, the mutants with low frequency of DSB are expected to have a mutation in the gene that is coding for the enzyme of interest. It is possible to identify this gene by comparing the genome sequence of the low DSB mutants with the wild-type cell.

It is important to identify enzymes that cause these chromosomal rearrangements because it would allow us to prevent related genetic disorders by interventions that inhibit these enzymes and reduces the amount of DSBs.

Background

A palindromic DNA is a sequence of DNA that repeats itself in opposite direction [Sinden, 1994]. Palindromes are perfect head-to-head inverted repeats, and quasi-palindromes are inverted repeats that are separated by a spacer [Sinden, 1994]. One strand of a palindromic DNA not only binds to the other strand of DNA, but it also can bind to itself, and therefore, it can form a hairpin structure when one strand is involved in intra-strand bind formation and a cruciform structure when both strands are involved [Sinden, 1994]. There are three factors that affect the rate of formation of hairpin and cruciform structures and their stability. These factors include length of the inverted repeats, length of the spacer (distance between the repeats) and composition of repeats

and/or spacer. The most unstable secondary structures are long, AT-rich sequences with absence of a spacer [Potaman and Sinden, 2004]. Palindromic sequences can potentially cause genome instability, which is due to secondary structures formation [Zhang et al., 2013]. In many eukaryotic cells, some DNA sequences have an ability to form cruciform or hairpin structures, which will increase the probability of DNA double strand breakage (DSB) [Zhang et al., 2013]. It has been shown in yeast, mice and human that formation of these secondary structures causes many gross chromosomal rearrangements (GCR) [Zhang et al, 2013]. Thalassemia and Emanuel syndrome are the two examples of genetic disorders in human that can be caused by the high frequency of GCR resulted from formation of secondary structures [Kato et al, 2012; Rooks et al., 2012]. Palindromes are also commonly found in cancer cells [Zhang et al, 2013]. It has been shown that nuclease attack is the main cause of palindromic DNA breakage [Zhang et al, 2013].

Even though the palindromes, their effects and diseases have been greatly studied so far, the main nuclease that causes these breakages and chromosomal rearrangements in eukaryotic cells is still unknown. Because palindromic DNAs are highly unstable, their study and detection in cancer cells requires special techniques.

In this study our goal is to identify the nucleases that break palindromic DNAs in yeast, with proposing projects that would help in understanding the unrevealed mechanisms of chromosomal rearrangements. Since during the evolution, DNA repair mechanisms have been conserved between yeast and human [Kakarougkas, 2014], the results of this study could be potentially extrapolated to humans. Some possible implications of this study are in prognosis of repeat-mediated diseases and in drug delivery industry.

CHAPTER 2

METHODS AND MATERIALS

Yeast Model

The yeast model that is used in this research possesses the following genes: *LYS2* gene that being disrupted by a *Alu* palindrome, *CAN1* gene that facilitates transportation of the toxic chemical L-canavanine from the drug-containing medium into cells, *ADE2* gene that if being inhibited or lost results to red pigment accumulation in cells (figure 2). Only cells with mutated or lost *CAN1* can survive on canavanine-containing medium. Cells without *ADE2* gene turn red in low adenine media, but the cells with *ADE2* gene remain white. This property of yeast model helps in distinguishing between the mutants that lose the arm telomeric to the palindrome versus the ones that only mutates *CAN1* gene.

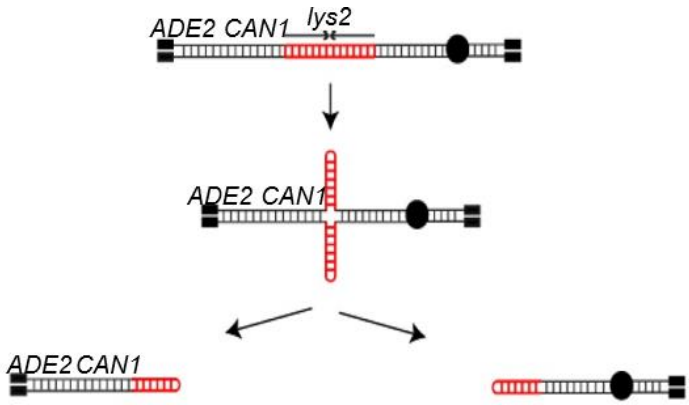

Figure 2, Yeast model

Identification of canavanine-sensitive mutants

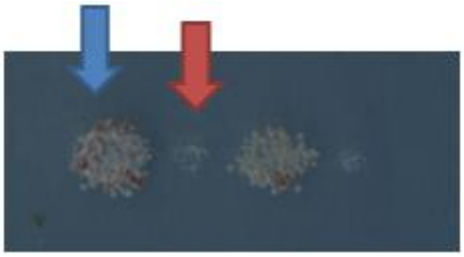

Figure 3, identification of GCR frequency of yeast mutants on canavenine media. The mutants with low GCR die on canavenine (red arrow) and the mutants with high GCR survive on canavenine (blue arrow)

Initially, cells that were mutated by mutagenic chemical EMS were obtained. The mutants were cultured on yeast extract peptone dextrose (YPD) media. Low concentration of cells were plated and spread using beads to assure growth of single colonies. Then the colonies were transferred onto canavanine media using replica plating method. Since canavanine is poisonous for yeast cells, the wild-type cells with *CAN1* gene die on canavanine media because they take in the canavanine, but the mutants that have lost the arm with *CAN1* gene survive on canavanine media since they are not able to transport the canavanine into the cells. The colonies that frequently gave rise to canavanine resistant red papillae were identified as high frequency GCR mutants and the ones that didn't produce canavanine resistant red papillae were identified as low frequency GCR mutants (figure 3). The corresponding colonies of low GCR mutants were identified on YPD plates and were recorded and preserved.

Identification of low DSB mutants

Pulsed Field Electrophoresis

The low GCR mutants were streaked out on YPD plates and inoculated in YPD liquid media. Hemocytometer was used for cell count. Agarose plugs were made with equal number of cells for each low GCR mutant, and therefore, each column of the electrophoresis gel had approximately the same amount of total DNA that allows for

accurate comparison between cells. Agarose gel was used for pulse field electrophoresis to separate the chromosomal DNA and the broken arm of cells. The ultra-violate (UV) picture of ethidium bromide stained gel was obtained to verify that DNA has been separated in gel and to observe the approximate DNA pattern on electrophoresis gel. A ladder marker was used to show an estimate of DNA size in the gel.

Southern Blotting

Southern blotting was used to identify possible low DSB mutants among canavanine intolerant mutants. The DNA from electrophoresis gel was transferred to a nylon membrane, and hybridized with a probe labelled with radioactive P32. The probe targets *HPA3* gene which is located at the region telomeric to *CAN1* gene. Radioactivity of the probe allows for accurate measurement of broken DNA. Then the radioactivity of the membrane was detected on an X-ray film. It is possible to compare the amount of DNA in each band by looking at intensity of radioactivity. The darker bands on the screen show higher amounts of DNA. The wild-type yeast is used as the control. The bands that show lighter signal intensity comparing to the wild-type represent mutants that possibly have lower DSB rates comparing to the wild-type.

CHAPTER 3

RESULTS AND DISCUSSION

234 low GCR mutants were identified out of which 8 could not be preserved because they were dead even on YPD. 2 of the mutants did not survive on YPD, but could be preserved from YPG plates (figure 4). All of the preserved mutants were tested by Southern blotting. The result of ethidium bromide gel electrophoresis showed that there is a DNA smear from agarose plugs and there are darker shades where the broken fragments are expected to be located (figure 5). The Southern blotting shows 3 bands (figure 6). One dark band at the top and two lighter bands at the bottom. The top band shows the unbroken chromosome V, and the lighter

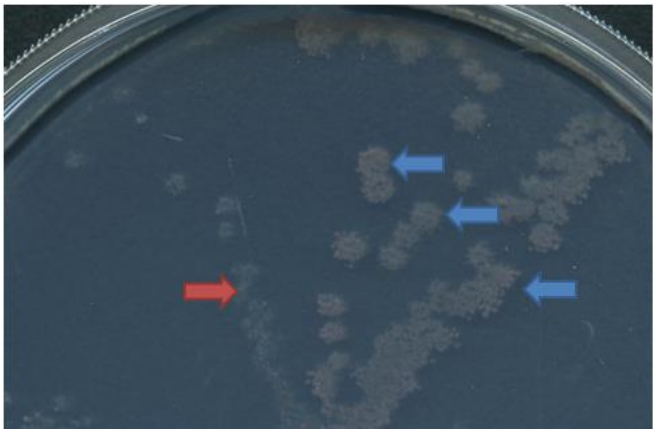

Figure 4, identification of canavanine sensitive mutants. Colonies that didn't grow on canavanine were canavanine sensitive (red arrow) and the ones that grew normally were canavenine resistant (blue arrow)

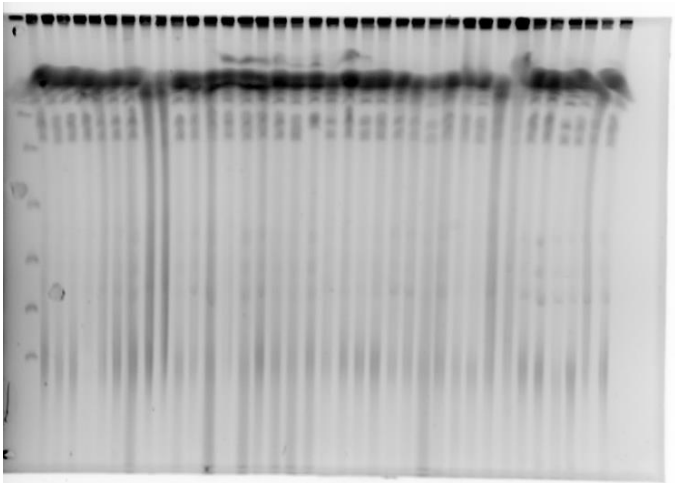

Figure 5, Agarose gel electrophoresis

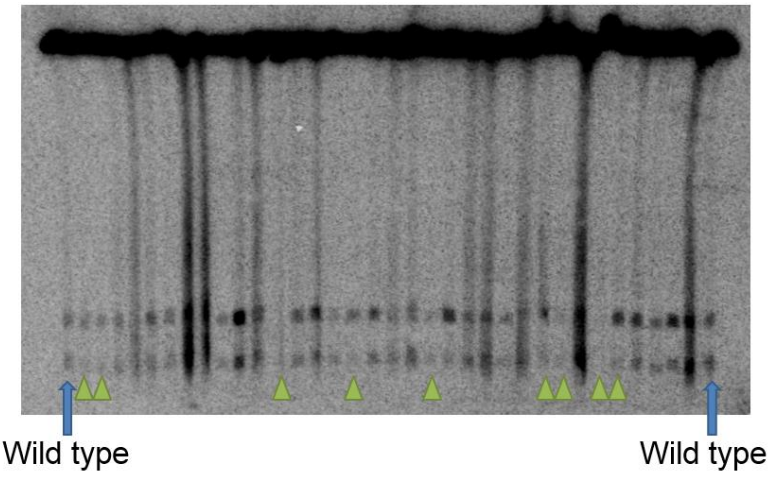

Figure 6, Southern blotting screen, the blue arrows show the wild type and green arrows show possible low DSB mutants

bands at the bottom show broken fragments. These broken fragments sometimes form double-sized fragments from replication. The band immediately above that of the broken DNA represents the replicated DNA. To identify low DSB mutants the lower bands were compared with the wild-type. The mutants that are marked with green arrow in figure 6 are possible low DSB mutants. The mutants that have a smear on Southern blotting screen which indicates the degradation of genomic DNA were not considered in this evaluation. 22 total possible low DSB mutants were found from Southern blotting experiment.

CHAPTER 5

CONCLUSION AND FUTURE WORK

The results from this research identified some mutants for further studies, but still more mutants should be tested to find more low DSB mutants. Also, the mutants identified from this project should be retested further for verification.

The next step is to compare the genome of low DSB mutants and the high DSB wild-type utilizing next generation DNA sequencing. The genes that are mutated among mutants are candidates to be the gene of interest.

For further verification, those genes could be deleted from wild-type cells and we can test those cells for GCR frequency. If a decrease in GCR is observed, it will be proved that the tested gene is indeed the gene responsible for coding the nuclease or a regulator that controls the nuclease that causes DSB at palindromic DNA.

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